#29
10/03

PATENT
071949-2104

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: BUECHLER et al.

Title: NOVEL METHODS FOR THE ASSAY OF TROPONIN I AND T AND COMPLEXES OF TROPONIN I AND T AND SELECTION OF ANTIBODIES FOR USE IN IMMUNOASSAYS

Appl. No.: 09/349,194

Filing Date: July 7, 1999

Examiner: Gail Gabel

Art Unit: 1641

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service's "Express Mail Post Office To Addressee" service under 37 C.F.R. § 1.10 on the date indicated below and is addressed to: Commissioner for Patents, PO Box 1450, Alexandria, Virginia 22313-1450.

EL990322920US (Express Mail Label Number) September 8, 2003 (Date of Deposit)

Germaine Sarda
(Printed Name)

(Signature)

APPEAL BRIEF

Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants (herein, "Appellants") hereby appeal the Final Rejection of claims 85-96, 102-106, and 119-133. This Appeal Brief is accompanied by the requisite fee set forth in 37 C.F.R. § 1.17(f). If this fee is incorrect or if any additional fees are due in this regard, please charge or credit our Deposit Account No. 50-0872 for the appropriate amount.

[illegible] 320.00 OP

Table of Contents

Table of Publications

Real Party in Interest

The real party in interest in this appeal is Biosite, Inc. (formerly Biosite Diagnostics, Inc.), which is the assignee of the present application.

Related Appeals and Interferences

Substantially the same issues presented in the present Appeal Brief are pending in related application 09/687,051. An appeal brief was submitted in this related application on August 20, 2003.

Status of Claims

On July 7, 2003, Appellants filed a Notice of Appeal from the Examiner's Action of March 7, 2003, making final the rejection of claims 85-96, 102-106, and 114-133. In that action, claims 85-96, 102-106, and 119-133 were finally rejected under 35 U.S.C. §112, first paragraph, for allegedly failing to satisfy the enablement requirement. The Examiner indicated that claims 134-142 would be allowable if written in independent form. Claims 1-84, 97-101, and 107-113 had been cancelled without prejudice.

The Examiner asserted in an Advisory Action mailed on August 18, 2003 that claims 114-118 and 139 were substantially duplicative of claims 102-106 and 138. The Advisory Action then maintained the rejection for lack of enablement only for claims 85-96, 102-106 and 119-133, apparently withdrawing this rejection with respect to claims 114-118. As discussed in the next section, Appellants disagree with the Examiner's assertion that claims 114-118 and 138 are substantial duplicates of claims 102-136 and 108, and Appellants believe that claims 114-118

should be considered subject to the rejection under 35 U.S.C. §112, first paragraph, and that claim 139 should be considered allowable.

Status of Amendments

In response to the Examiner's invitation to rewrite claims 134-142 in independent form, Appellants submitted an amendment after-final on July 7, 2003, adopting the Examiner's suggestions. The claims, as amended in this submission, are provided herewith as Appendix A for the convenience of the Board.

In an Advisory Action dated August 18, 2003, the amendment was entered by the Examiner. In responding to the amendment offered by Appellants after-final, the Examiner also objected for the first time to claims 114-118 and 139 as allegedly being duplicative of claims 102-106 and 138. To the contrary, claims 114-118 and 139 are directed to assays for determining the presence or amount of a free and complexed cardiac specific isoform of troponin, while claims 102-106 and 138 are directed to assays for determining the presence or amount of all free and complexed cardiac specific isoforms of troponin. An example within the scope of the former claims might be an assay that detects cardiac specific troponin I or cardiac specific troponin T in free and complexed forms; an example within the scope of the latter claims might be an assay that detects both cardiac specific troponin I and cardiac specific troponin T in free and complexed forms. Thus, the newly (and improperly) asserted objection of claims 114-118 and 139 as being duplicative of claims 102-106 and 138 is without basis and should be withdrawn.

Summary of The Invention

The present invention relates to antibodies that specifically bind to cardiac specific troponin isoforms in both free and complexed forms. In particular, the instant claims relate to

antibodies that are bind to each cardiac specific troponin form selected from the following group: (i) a free cardiac specific troponin isoform, (ii) the cardiac specific troponin isoform in binary complexes comprising another troponin component, and (iii) the cardiac specific troponin isoform in ternary complexes comprising two other troponin components.

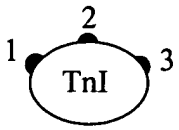
Troponin is a protein complex that is involved in regulating muscle contraction. The troponin complex is composed of three separate polypeptides, known as troponin I, troponin T, and troponin C. Specification, page 3, lines 29-30. Troponin exists in muscle mainly as a complex comprising all three troponin polypeptides – a so-called "ternary complex." *Id.*, page 5, lines 6-10. When muscle cells are damaged (such as occurs in skeletal muscle following exercise, or in cardiac muscle following a myocardial infarction), the contents of the muscle cells, including troponin, are released into the blood. For this reason, the ability to identify troponin released from heart muscle into the blood can provide a simple, rapid means of diagnosing myocardial infarction in a patient suspected of having suffered such an event. *Id.*, page 2, line 30, through page 3, line 28.

The amount of skeletal and smooth muscle in the body, however, is far in excess of the amount of heart muscle; thus, any assay designed to identify cardiac damage based upon the presence or amount of muscle components in the blood must be able to identify the cardiac components separate from the background of their skeletal and smooth muscle counterparts. Immunoassays generally rely on the use of antibodies that can specifically bind an analyte of interest for detection within a sample containing many other components. In the case of troponin, immunoassays have been developed to identify cardiac troponin through "cardiac specific" regions of troponin I and T; that is, regions that differ on cardiac troponin I and T in comparison to the same regions on skeletal or smooth muscle troponin I and T. *Id.*, page 3, lines 18-28. Such

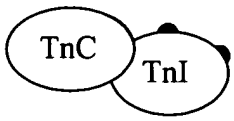
immunoassays can be formulated in a variety of ways, including "sandwich" immunoassays, "competitive binding" immunoassays, and other assays well known in the art. A signal from the immunoassay is detected and related to the presence or amount of the analyte.

As noted above, troponin exists in muscle mainly as a "ternary complex" comprising all three troponin polypeptides. *Id.*, page 5, lines 6-10. What was not understood prior to the present invention was that cardiac-specific troponin I and troponin T circulate in the blood in forms other than the ternery complex I/T/C. The inventors were the first to describe circulating free cardiac-specific isoforms (*i.e.*, troponin I and troponin T that are free of any other troponin polypeptides), and binary complexes (troponin I/T complex, troponin I/C complex, and/or troponin T/C complex), in addition to the ternary complexes known in the art. Furthermore, the "complex state" of troponin I and T may change over time in a patient, *e.g.*, due to binding of free cardiac-specific isoforms to other circulating troponin polypeptides. *Id.*, page 16, line 16, through page 17, line 19.

The present invention also recognized for the first time that immunoassays that fail to consider the "complex state" of cardiac-specific troponin may not detect all of the cardiac-specific isoform of interest. Taking troponin I as an example, this polypeptide contains certain antigenic sites that are "cardiac-specific." These regions may be present as numbered in this schematic drawing:

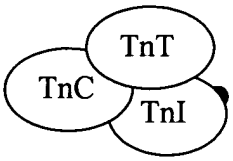


Binding of another troponin polypeptide, *e.g.*, troponin C, in a binary complex may obscure one or more of these cardiac-specific regions so that it is not accessible to antibodies directed to that site:



If antibodies directed to region 1 were used in a troponin I immunoassay, the troponin I concentration of the sample measured in the immunoassay may be incorrectly low, due to an inability to detect troponin I in complexes. Such aberrant assay results could end up in a misdiagnosis of patients. *Id.*, page 17, lines 26-30. On the other hand, selection of antibodies for use in an immunoassay that are directed to region 1 could be used to differentiate free troponin I from complexed troponin I forms, as such antibodies would not recognize troponin I in a binary complex.

Similarly, binding of the third troponin polypeptide, *e.g.*, troponin T, in a ternary complex may also obscure one or more of these cardiac-specific regions so that it is not accessible to antibodies directed to that site. Antigenic sites that remain available, however, may be used to recognize free troponin I, as well as the binary and ternary complexes:



Assays that can identify all of the cardiac specific troponin forms in a sample, regardless of the complex state, and assays that distinguish free cardiac-specific troponin isoforms from complexed isoforms or that distinguish the binary from ternary complexes of cardiac-specific tropinin isoforms, each provide clinically important data to caregivers. *Id.*, page 35, lines 17-26.

Issues

1. Whether claims 85-96, 102-106, and 114-133 meet the enablement standard of 35 U.S.C. §112, first paragraph.

Grouping of Claims

The claims on appeal are those pending after entry of the amendment after-final (Exhibit A). Of the rejected claims, claims 85-87 and 114-118 stand or fall together; claims 88-90 and 119-123 stand or fall together; claims 91-93 and 124-128 stand or fall together; claims 94-96 and 129-132 stand or fall together; claims 102-106 stand or fall together; claim 139 stands alone. The following summary indicates the differences between the claims.

Claims	Subject matter
(a) 85-87 and 114-118	assays for determining the presence or amount of a free and complexed cardiac specific isoform of troponin;
(b) 88-90 and 119-123	assays for determining the presence or amount of free and complexed cardiac specific isoforms of troponin;
(c) 91-93 and 124-128	assays for determining the presence or amount of free and complexed cardiac specific troponin I;
(d) 94-96 and 129-132	assays for determining the presence or amount of free and complexed cardiac specific troponin T;
(e) 102-106	assays for determining the presence or amount of all free and complexed cardiac specific isoforms of troponin.

Claim 139 depends from group (a) above.

Argument

35 U.S.C. § 112, First Paragraph, Enablement Rejection

The only issue on appeal is an alleged lack of enablement with regard to claims 85-96, 102-106, and 119-133. Appellants respectfully submit that the specification, which the Examiner acknowledges is enabling with regard to a pool of antibodies that specifically bind to each form of a cardiac specific troponin isoform of interest (*i.e.*, the free isoform, the isoform in binary complexes comprising another troponin component, and the isoform in ternary complexes comprising two additional troponin components), is also enabling with regard to a single antibody that binds to each of the recited troponin forms. The rejection is based on an unsupported and factually incorrect interpretation of the phrase "an antibody," and the examiner's personal opinion and unsupported conclusory statements regarding enablement. The Examiner also applies a standard for determining compliance with the enablement requirement that does not comport with the settled law. Because the enablement requirement of 35 U.S.C. §112, first paragraph, has been met, Appellants respectfully request that the rejection be withdrawn or reversed.

Applicable legal standard

The standard for determining enablement is whether the specification as filed provides sufficient information as to permit one skilled in the art to make and use the claimed invention. *United States v. Telectronics, Inc.*, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). The test of enablement is not whether experimentation is necessary, but rather whether any experimentation that is necessary is undue. *Id.* A considerable amount of experimentation is permitted, provided that it is merely routine, or provided that the specification provides a reasonable amount of

guidance with respect to the direction in which the experimentation should proceed. *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

The Examiner interprets the phrase "an antibody" to be equivalent to "a single monoclonal antibody" without any basis for that interpretation

In the Advisory Action mailed on August 18, 2003, the Examiner has for the first time indicated that the rejection is based on an interpretation of the phrase "an antibody" to mean "a single monoclonal antibody." *See, e.g.*, Paper No. 28, page 2, last paragraph ("the specification... does not reasonably provide enablement for an antibody, i.e. single monoclonal antibody, having specific binding for each and all of free cTnI, binary complex of cTn, and ternary complex of cTn") (emphasis added). The Examiner provides no basis for equating "an antibody" with "a single monoclonal antibody." Instead, this interpretation simply springs from the Examiner's pen. Appellants respectfully submit that this interpretation is both unsupported and factually incorrect.

The phrase "an antibody" as used in the claims does not equate to a single monoclonal antibody having a single binding specificity, as the Examiner apparently believes. For example, the instant specification states on page 8, lines 9-12, that an antibody may also be a polyclonal antibody. Moreover, the skilled artisan would readily acknowledge that monoclonal antibodies are but one type of antibody. For example, a polyclonal antibody is also "an antibody." *See, e.g., The Dictionary of Cell Biology*, 2nd Ed., Academic Press, San Diego, 1995 (polyclonal antibody: an antibody produced by several clones of B-lymphocytes as would be the case in a whole animal... whereas a monoclonal antibody is the product of a single clone of B-lymphocytes) (emphasis added), attached as Appendix B.

The fact that the Examiner is incorrect in equating the phrase "an antibody" to "a single

monoclonal antibody" is further emphasized by a declaration of one of skill in the art, Dr. Kenneth F. Buechler, provided to the Examiner during prosecution of the present application. In paragraph 9 of that declaration, Dr. Buechler stated that the phrase "an antibody" does not refer to a single molecule of antibody, but rather is understood in the art to refer to a single population of antibody. Thus, a "polyclonal antibody" may refer to a population of individual antibody molecules having a variety of specificities; likewise, a "monoclonal antibody" may refer to a population of individual antibody molecules having a common specificity.

It is axiomatic that any enablement analysis must be based upon a correct interpretation of the claims at issue. *See, e.g.*, MPEP § 2164.04 (before any analysis of enablement can occur, it is necessary for the examiner to construe the claims); MPEP § 2111 (the interpretation of the claims must be consistent with the interpretation that those skilled in the art would reach). Because the Examiner begins the enablement analysis with an interpretation of the claims that is not supported by the specification or consistent with that which one skilled in the art would reach, the rejection under 35 U.S.C. §112, first paragraph, is fatally flawed from its very inception.

The rejection ignores the evidence of record in favor of the Examiner's personal opinion

The rejection is also premised on the Examiner's unsupported assertion that, while the specification is enabling with regard to a pool of antibodies that specifically bind to each form of a cardiac specific troponin isoform (*i.e.*, free isoform, the isoform in binary complexes comprising another troponin component, and the isoform in ternary complexes comprising two additional troponin components), the specification is not enabling with regard to a single antibody that binds to each of the recited troponin forms. *See, e.g.*, Paper No. 25, paragraph page 2. As discussed above, the claims are not limited to any particular type of antibody, and would

encompass both monoclonal antibodies and polyclonal antibodies. It is well known that a polyclonal antibody by definition is a mixture of different monoclonal antibodies. Thus, the examiner's view that the instant specification is enabling with respect to a pool of antibodies requires that a polyclonal antibody of the claims would similarly be enabled. Appellants, therefore, understand that the Examiner's enablement rejection is based on an interpretation of the phrase "an antibody" to mean "a single monoclonal antibody."

During prosecution of the instant claims, Appellants provided a declaration of one of skill in the art, Dr. Kenneth F. Buechler, as evidence of enablement of the claimed invention. In the declaration, Dr. Buechler provided a reasoned scientific explanation as to why the skilled artisan, using the specification as a guide and only routine methods that are well known in the art, could practice the instantly claimed invention. The Examiner has not attempted to rebut the conclusions provided by Dr. Buechler in the declaration, or suggested that the reasoning underpinning these conclusions is not scientifically sound. Instead, rather than consider this evidence on its merits, the Examiner has improperly dismissed the declaration on the basis of an improper evidentiary standard, which requires actual data to prove enablement. ("Applicant fails to provide evidentiary showing such as in the form of data, that supports generation [of the antibodies of the claims])." Paper No. 25, page 9.

Rather than consider the Buechler declaration, the Examiner denigrates it as "prophetic" and "speculative," (*see, e.g.*, Paper No. 28, page 4) without any reasoning as to why the declaration should not be believed. Appellants respectfully submit that the Buechler declaration is evidence that must be considered; in contrast, the personal opinion of the Examiner is not. By dismissing Appellants' evidence without proper consideration of its weight, the Examiner has failed to weigh the evidence as a whole, a consideration that is fundamental in any determination

of enablement. As stated in MPEP § 2164.05, a declaration or affidavit is, itself, evidence that must be considered, and the evidence need not be conclusive, but merely convincing to the skilled artisan.

The Examiner then compounds this failure by applying an improper legal standard for judging compliance with enablement requirement, asserting that, regardless of the evidence of record, only data showing that the antibodies of the claims have been generated is sufficient to demonstrate enablement. Appellants respectfully submit that this is not a correct legal standard. As noted in *In re Wands*, the presence of working examples is one consideration in an enablement analysis; it is not the single determinative consideration as the Examiner apparently believes. [1]

In effect, the Examiner has interpreted the claims to remove subject matter that the Examiner might consider enabled (*e.g.*, by improperly equating the phrase "an antibody" to "a single monoclonal antibody," use of a polyclonal antibody need not be considered). Then, the Examiner ignores a presumptively accurate specification and declaratory evidence from one of skill in the art, in favor of the Examiner's own unsupported opinion that the claims are not enabled, coupled to an enablement standard that requires hard data of reduction to practice in order to convince the Examiner otherwise. This rejection is maintained despite the fact that all starting materials to produce the antibodies are readily available, and all of the methods required to produce the antibodies are routine in the art. As stated in MPEP § 2164.04, "it is incumbent on the Patent Office... to explain why it doubts any statement in a disclosure, and to back up its assertions of its own with acceptable evidence or reasoning.... Otherwise, there would be no

[1] As discussed hereinafter, the Examiner is also incorrect in asserting that no evidence has been provided that such antibodies have been generated. *See*, section entitled "*Presence of Working Examples," infra.*

need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." The Examiner has simply not performed this task, which, like consideration of the evidence as a whole, is fundamental in any determination of enablement. As such, the Examiner has not established any reasonable basis for questioning the enablement of the claims.

When properly considered, the evidence of record demonstrates that the claims satisfy the enablement requirement

Appellants respectfully submit that the following analysis of the factors set forth in *In re Wands* demonstrates that the rejected claims meet the enablement standard of 35 U.S.C. § 112. In contrast, while the Examiner "goes through the motions" of analyzing the *Wands* factors, that analysis relies on personal opinion and unsupported conclusory statements and is applied to a standard for determining compliance with the enablement requirement that does not comport with the settled law.

Nature of the invention

The claims at issues are directed to assay methods for determining the presence or amount of free and complexed cardiac specific troponin isoform(s). These assay methods comprise performing an immunoassay using one or more antibodies that specifically bind each form of the troponin isoform(s) of interest: (i) free cardiac specific isoform, (ii) cardiac specific isoform in a binary complex comprising another troponin component, and (iii) cardiac specific isoform in a ternary complex comprising two other troponin components.

In contrast, the Examiner continues to mischaracterize the invention by maintaining that it is "directed to a method for determining the presence or amount of all free and complexed isoforms of [cardiac troponin] using a cocktail of antibodies having specific binding for each and all of free, binary complex, and ternary complex isoforms of [cardiac troponin]." (Paper No. 25,

page 3, emphasis added). The Examiner continues to make this characterization despite the fact that the specification as filed states explicitly that the use of a cocktail of antibodies for this purpose is but one embodiment of the instant invention. For example, the specification explicitly states:

> "[t]he immunoassay may be formulated with a cocktail of antibodies to bind all the troponin complexes and the free troponin I and T. Alternatively, the immunoassay can be formulated with specific antibodies that recognize epitopes of the troponin I and T in the complexes and also the unbound troponin I and T. A preferred immunoassay for troponin I or T involves conjugation of an antibody or a cocktail of antibodies to a label or signal generator to form an antibody conjugate(s), which are capable of binding to cardiac specific regions of the troponin complexes of troponin I or T and to unbound troponin I or T.

Page 24, line 21, through page 25, line 3. This section and others in the specification clearly indicate that the invention is not limited to "cocktails" of antibodies, as the Examiner contends. Furthermore, only certain claims refer to determining the presence or amount of all free and complexed isoforms, which the Examiner inexplicably considers the "nature of the invention" as a whole.

Thus, the Examiner begins the enablement analysis with a biased personal opinion of the meaning of the phrase "an antibody," and a biased personal opinion of the "nature of the invention." In so doing, the Examiner presupposes what conclusion should be reached regarding enablement, and then, improperly, finds agreement with that conclusion. Appellants respectfully submit that the Examiner's statement of the "nature of the invention," like the Examiner's interpretation of the meaning of the phrase "an antibody," is without support of any evidence of record.

State of the prior art

The Examiner has not disagreed with Appellants' view that the prior art fails to disclose any antibodies that specifically bind each form of the troponin isoform(s) of interest, *i.e.*, free cardiac specific isoform, cardiac specific isoform in a binary complex comprising another troponin component, and cardiac specific isoform in a ternary complex comprising two other troponin components; but that methods for producing antibodies to an antigen of interest are well established in the art.

Level of one of ordinary skill

The Examiner has not disagreed with Appellants that the level of skill in the art of antibody preparation is high, and that methods for producing antibodies to an antigen of interest are considered routine by those of skill in the art. With regard to monoclonal antibodies specifically, the court in *In re Wands* acknowledged that methods for obtaining and screening monoclonal antibodies were well known even as of 1980, some 15 years before the original filing date of the present application. *See* 8 USPQ2d at 1406. Thus, all of the methods needed to practice the invention of the claims at issue have long been readily available to those of ordinary skill in the art.

Predictability in the art

Appellants respectfully submit that the starting materials (the troponin polypeptides) necessary for generation of the recited antibodies are readily available to the skilled artisan. Coupled with the fact that methods for generating both monoclonal and polyclonal antibodies have long been well known and considered routine in the art, the skilled artisan could predictably practice the claimed invention using the instant specification as a guide. As further evidence of

this predictability, Appellants submitted the declaration of Dr. Kenneth Buechler, describing why the skilled artisan would reasonably believe that antibodies that specifically bind each form of the troponin isoform(s) of interest could be obtained.

As discussed in the Buechler declaration, cardiac specific troponin isoforms contain various antigenic sites. Because certain antigenic sites may remain available for antibody binding regardless of the complex state of troponin isoform, these sites may be used to bind a free cardiac specific troponin isoform of interest, as well as the cardiac specific troponin isoform in binary and ternary complexes. Nothing of record, other than the Examiner's bare personal view, contradicts this reasoned scientific conclusion.

Moreover, the examples described in the instant invention confirm the accuracy of the scientific basis for Dr. Buechler's statements. For example, Example 23, on page 87, line 31, through page 88, line 2, of the specification, describes the selection of antibodies that bind to both free troponin I and to troponin I in a ternary complex with troponin C and troponin T. According to Dr. Buechler, the skilled artisan would understand that antibodies which bind to free troponin I and to troponin I/C/T ternary complex would also be expected to bind to the binary complex of troponin I and troponin C, because the antigenic site on troponin I, which is available for binding in the ternary complex, would also be expected to be available in the simpler binary complex. *See, e.g.*, drawing on page 3, of Buechler declaration.

Furthermore, while the declaration of Dr. Buechler describes why the skilled artisan would reasonably believe that even a monoclonal antibody could be produced having the requisite specificity, the instant claims are not limited to a monoclonal antibody having a single binding specificity. Instead, as discussed above, "an antibody" may also be "a polyclonal antibody." Such a polyclonal antibody would be considered equivalent to the "antibody pools"

which the Examiner acknowledges meet the enablement standard. The skilled artisan would understand that a polyclonal antibody that binds each form of a cardiac specific troponin isoform of interest (*i.e.*, the free isoform, the isoform in binary complexes comprising another troponin component, and the isoform in ternary complexes comprising two additional troponin components) could readily be obtained by immunization of an animal with each of these forms as described in the specification, *e.g.*, on page 21, lines 3-32, and isolating the antibodies produced.

In contrast to the substantial evidence of predictability provided by Appellants, the Examiner offers only the bare conclusory statement that "there is no predictability" in the art. *See, e.g.*, Paper No. 25, page 3, last paragraph. Appellants cannot discern any basis for this statement, particularly in view of substantial evidence to the contrary already of record in the case. The Examiner's unsupported conclusion fails to establish a reasonable basis for questioning the enablement provided in the specification. As stated in MPEP § 2164.04, "it is incumbent on the Patent Office... to explain why it doubts any statement in a disclosure, and to back up its assertions of its own with acceptable evidence or reasoning.... Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure."

Considering the objective evidence of record in its entirety, Appellants respectfully submit that the skilled artisan would acknowledge that it would be predictable that the antibodies of the claims at issue could be obtained using the specification as a guide and only routine immunological methods.

The amount of direction or guidance present

The instant specification provides extensive guidance as to how antigens should be prepared, and how antibodies should be screened, in order to obtain antibodies that specifically bind each form of the troponin isoform(s) of interest. *See, e.g.*, specification, page 21, line 3, through page 24, line 3; Example 22 beginning on page 82; and Example 23, beginning on page 87.

In contrast, the Examiner offers only the conclusory statement that "the specification fails to provide any guidance to enable the claimed method to make and use an antibody that specifically binds all of the free, binary and ternary complexed isoforms of cTn." *See, e.g.*, Paper No. 25, page 4, first paragraph. Again, such a conclusory statement does not establish a reasonable basis for questioning the enablement provided in the specification. Moreover, the Examiner's response to Appellants' evidence (*i.e.*, "nowhere in the specification specifically shows... any generation and selection [of such antibodies]," paper No. 25, page 10, first paragraph) seeks yet again to apply the improper standard that only data showing that the antibodies of the claims have been generated is sufficient to demonstrate enablement. The question is not whether such antibodies have been generated, as the Examiner suggests; rather, the question is whether the specification enables such antibodies to be generated without undue experimentation. Furthermore, as discussed in the following section of this submission, the Examiner is incorrect that no such antibodies are demonstrated in the specification.

Considering the objective evidence of record in its entirety, Appellants respectfully submit that the skilled artisan would acknowledge that the specification provides extensive guidance for making and using the claimed invention.

Presence of working examples

Example 23 of the instant specification describes antibodies and assays that measure both free and ternary complexes of troponin I. Because these antibodies rely on formation of a sandwich of (labeled antibody)-(analyte)-(biotinylated antibody)-(avidin solid phase) for development of an assay signal, the skilled artisan would acknowledge that both the biotinylated and labeled antibodies must bind to both free and ternary troponin I complexes. This means that the antigenic site on troponin I (to which the antibody binds) was accessible to antibody even when troponin I was bound to both troponin T and troponin C in the ternary complex. Similarly, the example beginning on page 76 shows equivalent assays that measure both free and ternary complexes of troponin T. Again, the antigenic site on troponin T (to which the antibody binds) was accessible to antibody even when troponin T was bound to both troponin I and troponin C in the ternary complex.

As discussed in the Beuchler declaration, the skilled artisan would understand from this evidence that antibodies which bind to free and ternary complexes of a troponin isoform would also be expected to bind to binary complexes of the isoform, because sites that are free in the ternary complex would be expected to remain free in the simpler binary complex. *See, e.g.*, drawing on page 3, of Buechler declaration. Thus, one of ordinary skill in the art would believe that the instant specification provides working examples of antibodies that specifically bind each of the free and complexed forms of the troponin isoform(s) of interest.

The Examiner's reply indicates that the Examiner disagrees with this reasoning, which is based on evidence of record and sound scientific principles. But the Examiner provides no evidence or reasoning that is inconsistent with Appellants' evidence. Instead, the Examiner again simply asserts that nowhere in the examples is such an antibody "specifically" provided. Paper

No. 25, page 11, first full paragraph. This is yet another application of the Examiner's improper standard for proof of enablement.

Considering the objective evidence of record in its entirety, the skilled artisan would acknowledge that working examples of antibodies that specifically bind each form of the troponin isoform(s) of interest are provided by the instant specification.

Quantity of experimentation necessary

As described in detail in the Buechler declaration and in Appellants' prior responses, the specification provides detailed methods that utilize readily available starting materials for preparing and identifying antibodies that specifically bind each form of a cardiac specific troponin isoform of interest (*i.e.*, the free isoform, the isoform in binary complexes comprising another troponin component, and the isoform in ternary complexes comprising two additional troponin components). And, as discussed above, the Examiner does not disagree that methods for producing antibodies generally have long been well known and considered routine by those of skill in the art. Taken together, these facts lead to the inescapable conclusion that the quantity of experimentation necessary is not undue. *See, e.g., In re Wands*, 8 USPQ2d at 1404 (experimentation is not undue if it is merely routine).

In contrast, the totality of the Examiner's analysis on this issue is a conclusory view that "it would require undue amount of experimentation for the skilled artisan to make and use the method as claimed." Paper No. 25, page 4, third paragraph. Again, such an assertion, unsupported by any evidence or reasoning of record, cannot establish a lack of enablement. Moreover, in response to Appellants' arguments in this regard, the Examiner again relies on an assertion that nowhere in the examples is such an antibody demonstrated by actual data (Paper

No. 25, page 11, second full paragraph), and disregards whether the evidence of record implicitly indicates that such antibodies have been described. The question is whether the specification enables such antibodies to be generated without undue experimentation, and not whether such antibodies have been demonstrated by unambiguous data, as the Examiner apparently believes. MPEP § 2164.05.

The instant claims meet the enablement standard of 35 U.S.C. § 112, first paragraph

In view of the objective evidence of record, and the foregoing analysis of the factors set forth in *In re Wands*, Appellants respectfully submit that that the present claims meet the enablement standard of 35 U.S.C. § 112, first paragraph. The Examiner's opinion to the contrary is not based upon objective evidence of record, but instead is based on personal opinion, unsupported conclusory statements, and application of a standard for determining compliance with the enablement requirement that does not comport with the settled law.

Conclusion

For the reasons discussed above, Appellants respectfully submit that all the claims are in condition for allowance, and respectfully request that the rejections be withdrawn or reversed, and that the claims be allowed to issue.

Respectfully submitted,

Date September 8, 2003

By _____

Barry S. Wilson
Attorney for Applicant
Registration No. 39,431

FOLEY & LARDNER
Customer Number: 30542
30542
PATENT TRADEMARK OFFICE
Telephone: (858) 847-6722
Facsimile: (858) 792-6773

Appendix A: Text of the Claims as Amended in Submission Dated July 7, 2003

1-84 (Previously cancelled)

85. (Previously presented) An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds said free cardiac specific isoform of troponin, and which specifically binds said cardiac specific isoform of troponin in a binary complex comprising one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds said cardiac specific isoform of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoform of troponin, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific isoform of troponin; (ii) troponin complexes which do not comprise said cardiac specific isoform of troponin; or (iii) a combination of (i) and (ii), and wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoform of troponin in said sample.

86. (Previously presented) An assay according to claim 85, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

87. (Previously presented) An assay according to claim 85, wherein said signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoform of troponin is at least a factor of five greater than said signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific isoform of troponin; (ii) troponin complexes which do not comprise said cardiac specific isoform of troponin; or (iii) a combination of (i) and (ii).

88. (Previously presented) An assay for determining the presence or amount of free and complexed cardiac specific isoforms of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin I and cardiac specific troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoforms of troponin, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific isoforms of troponin; (ii) troponin complexes which do not comprise said cardiac specific isoforms of troponin; or (iii) a combination of (i) and (ii), and wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoforms of troponin in said sample.

89. (Previously presented) An assay according to claim 88, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

90. (Previously presentetd) An assay according to claim 88, wherein said signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoforms of troponin is at least a factor of five greater than said signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific isoforms of troponin; (ii) troponin complexes which do not comprise said cardiac specific isoforms of troponin; or (iii) a combination of (i) and (ii).

91. (Previously presented) An assay for determining the presence or amount of free and complexed cardiac specific troponin I in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin I, and which specifically binds to cardiac specific troponin I in a complex comprising at least one other troponin component selected from the group consisting of troponin C and troponin T, and which specifically binds to cardiac specific troponin I in a ternary complex comprising troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin I, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific troponin I; (ii) troponin complexes which do not comprise said cardiac specific troponin I; or (iii) a combination of (i) and (ii), and wherein said detectable signal is related to the presence or amount of said free and complexed cardiac specific troponin I in said sample.

92. (Previously presented) An assay according to claim 91, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

93. (Previously presented) An assay according to claim 91, wherein said signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin I is at least a factor of five greater than said signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific troponin I; (ii) troponin complexes which do not comprise said cardiac specific troponin I; or (iii) a combination of (i) and (ii).

94. (Previously presented) An assay for determining the presence or amount of free and complexed cardiac specific troponin T in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin T, and which specifically binds to cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin C and troponin I, and which specifically binds to cardiac specific troponin T in a ternary complex comprising troponin C and troponin I; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin T, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific troponin T; (ii) troponin complexes which do not comprise said cardiac specific troponin T; or (iii) a combination of (i) and (ii), and wherein said detectable signal is related to the presence or amount of said free and complexed cardiac specific troponin T in said sample.

95. (Previously presented) An assay according to claim 94, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

96. (Previously presented) An assay according to claim 94, wherein said signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin T is at least a factor of five greater than said signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific troponin T; (ii) troponin complexes which do not comprise said cardiac specific troponin T; or (iii) a combination of (i) and (ii).

97-101 (Previously cancelled)

102. (Previously presented) An assay for determining the presence or amount of all free and complexed cardiac specific isoforms of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds all free cardiac specific isoforms of troponin, and which specifically binds all cardiac specific isoforms of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds all cardiac specific isoforms of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoforms of troponin, wherein said signal is related to the

presence or amount of all free and complexed cardiac specific isoforms of troponin in said sample.

103. (Previously presented) An assay according to claim 102, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

104. (Previously presented) An assay according to claim 102, wherein said signal is approximately equal for equal amounts of all cardiac specific isoforms of troponin.

105. (Previously presented) An assay according to claim 102, wherein said signal is within 20% for equal amounts of all cardiac specific isoforms of troponin.

106. (Previously presented) An assay according to claim 102, wherein said signal is within a factor of 2 for equal amounts of all cardiac specific isoforms of troponin.

107-113 (Previously cancelled)

114. (Previously added) An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds said free cardiac specific isoform of troponin, and which specifically binds said cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds said cardiac specific isoform of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoform of troponin, wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoforms of troponin in said sample.

115. (Previously presented) An assay according to claim 114, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

116. (Previously presented) An assay according to claim 114, wherein a signal detected from said immunoassay for an amount of said free cardiac specific isoform of troponin is approximately equal to a signal detected from said immunoassay for an equal amount of said complexed cardiac specific isoform of troponin.

117. (Previously presented) An assay according to claim 114, wherein a signal detected from said immunoassay for an amount of said free cardiac specific isoform of troponin is within 20% of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific isoform of troponin.

118. (Previously presented) An assay according to claim 114, wherein a signal detected from said immunoassay for an amount of said free cardiac specific isoform of troponin is within a factor of 2 of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific isoform of troponin.

119. (Previously amended) An assay for determining the presence or amount of free and complexed cardiac specific isoforms of troponin in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin I and free cardiac specific troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific isoforms of troponin, wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoforms of troponin in said sample.

120. (Previously presented) An assay according to claim 119, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

121. (Previously presented) An assay according to claim 119, wherein a signal detected from said immunoassay for an amount of said free cardiac specific isoforms of troponin is approximately equal to a signal detected from said immunoassay for an equal amount of said complexed cardiac specific isoforms of troponin.

122. (Previously presented) An assay according to claim 119, wherein a signal detected from said immunoassay for an amount of said free cardiac specific isoforms of troponin is within 20% of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific isoforms of troponin.

123. (Previously presented) An assay according to claim 119, wherein a signal detected from said immunoassay for an amount of said free cardiac specific isoforms of troponin is within a factor of 2 of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific isoforms of troponin.

124. (Previously presented) An assay for determining the presence or amount of free and complexed cardiac specific troponin I in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin I, and which specifically binds to cardiac specific troponin I in a complex comprising at least one other troponin component selected from the group consisting of troponin C and troponin T, and which specifically binds to cardiac specific troponin I in a ternary complex comprising troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin I, wherein said signal is related to the presence or amount of said free and complexed cardiac specific troponin I in said sample.

125. (Previously presented) An assay according to claim 124, wherein said patient sample is selected from the group consisting of a blood sample, a serum sample, and a plasma sample.

126. (Previously presented) An assay according to claim 124, wherein a signal detected from said immunoassay for an amount of said free cardiac specific troponin I is approximately equal

to a signal detected from said immunoassay for an equal amount of said complexed cardiac specific troponin I.

127. (Previously presented) An assay according to claim 124, wherein a signal detected from said immunoassay for an amount of said free cardiac specific troponin I is within 20% of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific troponin I.

128. (Previously presented) An assay according to claim 124, wherein a signal detected from said immunoassay for an amount of said free cardiac specific troponin I is within a factor of 2 of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific troponin I.

129. (Previously presented) An assay for determining the presence or amount of free and complexed cardiac specific troponin T in a patient sample, said assay comprising:

performing an immunoassay with an antibody which specifically binds to free cardiac specific troponin T, and which specifically binds to cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I and troponin C, and which specifically binds to cardiac specific troponin I in a ternary complex comprising troponin I and troponin C; and

detecting a signal from said immunoassay resulting from said antibody binding said free and complexed cardiac specific troponin T, wherein said signal is related to the presence or amount of said free and complexed cardiac specific troponin T in said sample.

130. (Previously presented) An assay according to claim 129, wherein said patient sample is selected from the group consisting of a blood sample a serum sample, and a plasma sample.

131. (Previously presented) An assay according to claim 129, wherein a signal detected from said immunoassay for an amount of said free cardiac specific troponin T is approximately equal to a signal detected from said immunoassay for an equal amount of said complexed cardiac specific troponin T.

132. (Previously presented) An assay according to claim 129, wherein a signal detected from said immunoassay for an amount of said free cardiac specific troponin T is within 20% of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific troponin T.

133. (Previously presented) An assay according to claim 129, wherein a signal detected from said immunoassay for an amount of said free cardiac specific troponin T is within a factor of 2 of a signal detected from said immunoassay for an equal amount of said complexed cardiac specific troponin T.

134. (Presently Amended) An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 85, wherein said antibody~~ ~~is~~ an antibody cocktail which specifically binds said free cardiac specific isoform of troponin, and which specifically binds said cardiac specific isoform of troponin in a binary complex comprising one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds said cardiac specific isoform of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific isoform of troponin, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific isoform of troponin; (ii) troponin complexes which do not comprise said cardiac specific isoform of troponin; or (iii) a combination of (i) and (ii), and wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoform of troponin in said sample.

135. (Presently Amended) An assay for determining the presence or amount of free and complexed cardiac specific isoforms of troponin in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 88, wherein said antibody is~~ an antibody cocktail which specifically binds to free cardiac specific troponin I and cardiac specific troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific isoforms of troponin, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific isoforms of troponin; (ii) troponin complexes which do not comprise said cardiac specific isoforms of troponin; or (iii) a combination of (i) and (ii), and wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoforms of troponin in said sample.

136. (Presently Amended) An assay for determining the presence or amount of free and complexed cardiac specific troponin I in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 91, wherein said antibody is~~ an antibody cocktail which specifically binds to free cardiac specific troponin I, and which specifically binds to cardiac specific troponin I in a complex comprising at least one other troponin component selected from the group consisting of troponin C and troponin T, and which specifically binds to cardiac specific troponin I in a ternary complex comprising troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific troponin I, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific troponin I; (ii) troponin complexes which do not comprise said cardiac specific troponin I; or (iii) a combination of (i) and (ii), and wherein said

detectable signal is related to the presence or amount of said free and complexed cardiac specific troponin I in said sample.

137. (Presently Amended) An assay for determining the presence or amount of free and complexed cardiac specific troponin T in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 94, wherein said antibody is~~ an antibody cocktail which specifically binds to free cardiac specific troponin T, and which specifically binds to cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin C and troponin I, and which specifically binds to cardiac specific troponin T in a ternary complex comprising troponin C and troponin I; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific troponin T, wherein said signal is at least a factor of two larger than a signal resulting from said antibody binding to an equal number of (i) free troponin components which are not said cardiac specific troponin T; (ii) troponin complexes which do not comprise said cardiac specific troponin T; or (iii) a combination of (i) and (ii), and wherein said detectable signal is related to the presence or amount of said free and complexed cardiac specific troponin T in said sample.

138. (Presently Amended) An assay for determining the presence or amount of all free and complexed cardiac specific isoforms of troponin in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 102, wherein said antibody is~~ an antibody cocktail which specifically binds all free cardiac specific isoforms of troponin, and which specifically binds all cardiac specific isoforms of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds all cardiac specific isoforms of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific isoforms of troponin, wherein said signal is related to the presence or amount of all free and complexed cardiac specific isoforms of troponin in said sample.

139. (Presntly Amended) An assay for determining the presence or amount of a free and complexed cardiac specific isoform of troponin in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 114, wherein said antibody is~~ an antibody cocktail which specifically binds said free cardiac specific isoform of troponin, and which specifically binds said cardiac specific isoform of troponin in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds said cardiac specific isoform of troponin in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific isoform of troponin, wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoforms of troponin in said sample.

140. (Presently Amended) An assay for determining the presence or amount of free and complexed cardiac specific isoforms of troponin in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 119, wherein said antibody is~~ an antibody cocktail which specifically binds to free cardiac specific troponin I and free cardiac specific troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I, troponin C and troponin T, and which specifically binds to cardiac specific troponin I and cardiac specific troponin T in a ternary complex comprising two other troponin components selected from the group consisting of troponin I, troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific isoforms of troponin, wherein said signal is related to the presence or amount of said free and complexed cardiac specific isoforms of troponin in said sample.

141. (Presently Amended) An assay for determining the presence or amount of free and complexed cardiac specific troponin I in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 124, wherein said antibody is~~ an antibody cocktail which specifically binds to free cardiac specific troponin I, and which specifically binds to cardiac specific troponin I in a complex comprising at least one other troponin component selected from the group consisting of troponin C and troponin T, and which specifically binds to cardiac specific troponin I in a ternary complex comprising troponin C and troponin T; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific troponin I, wherein said signal is related to the presence or amount of said free and complexed cardiac specific troponin I in said sample.

142. (Presently Amended) An assay for determining the presence or amount of free and complexed cardiac specific troponin T in a patient sample, said assay comprising:

performing an immunoassay with ~~An assay according to claim 129, wherein said antibody is~~ an antibody cocktail which specifically binds to free cardiac specific troponin T, and which specifically binds to cardiac specific troponin T in a complex comprising at least one other troponin component selected from the group consisting of troponin I and troponin C, and which specifically binds to cardiac specific troponin I in a ternary complex comprising troponin I and troponin C; and

detecting a signal from said immunoassay resulting from said antibody cocktail binding said free and complexed cardiac specific troponin T, wherein said signal is related to the presence or amount of said free and complexed cardiac specific troponin T in said sample.

The Dictionary of

CELL BIOLOGY

Second Edition

EDITED BY

J. M. Lackie and J. A. T. Dow

Yamanouchi Research Institute, Littlemore Hospital, Oxford OX4 4XN, UK

and

Laboratory of Cell Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

AUTHORS

S. E. Blackshaw[1]
C. T. Brett
A. S. G. Curtis
J. A. T. Dow
J. G. Edwards[2]
J. M. Lackie
A. J. Lawrence
G. R. Moores

[1] Second edition only
[2] First edition only

ACADEMIC PRESS

Harcourt Brace & Company, Publishers
London San Diego New York
Boston Sydney Tokyo Toronto

Appendix B

ACADEMIC PRESS LIMITED
24–28 Oval Road
London NW1 7DX

US Edition published by
ACADEMIC PRESS INC.
San Diego, CA 92101

First published 1989

This book is printed on acid-free paper

A CIP record for this book is available from the British Library

Cased edition ISBN 0-12-432562-9
Paperback edition ISBN 0-12-432563-7

Cover illustration

NIH 3T3 cells were incubated in our fixable dye **MitoTracker™ CMXRos** (M-7512), which stains mitochondria red, and then treated with aldehyde fixatives. After the fixed cells were permeabilized with acetone, they were stained with **BODIPY® FL phallacidin** (B-607), which labels actin green, and with **POPO™-1** (P-3580), which labels nuclei blue. This photomicrograph was obtained with a single exposure through the Omega® Optical triple-band filter set (O-5855), available directly from Molecular Probes. Photo contributed by Ian Clements and Sam Wells, Molecular Probes.

Typeset by Fakenham Photosetting Limited, Fakenham, Norfolk
Printed and bound in Great Britain by Mackays of Chatham, PLC, Chatham, Kent

in moving cells, having a distinct front and rear. Some cells seem to show multiple axes of polarity (which will hinder forward movement).

polarisation microscopy Any form of microscopy capable of detecting birefringent objects. Usually performed with a polarising element below the stage to produce plane-polarised light, and an analyser that is set to give total extinction of the background, and thus to detect any birefringence.

pole cell A cell at or near the animal or vegetal pole of an embryo.

pole fibres Microtubules inserted into the pole regions of the mitotic spindle (each pole is the product of the division of the centrioles and constitutes a ***microtubule organising centre***.

polehole *Drosophila* homologue of the ***raf oncogene***.

poliovirus A member of the enterovirus group of *Picornaviridae* that causes poliomyelitis.

pollen mother cell A diploid plant cell that forms four ***microspores*** by meiosis; the microspores give rise to pollen grains in seed plants.

poly (ADP-ribose) polymerase (PARP) Enzyme that catalyses the attachment of ADP-ribose units to various nuclear proteins. This ***post-translational modification*** of proteins is dependent on DNA and appears to be involved in the regulation of various cellular processes such as differentiation, proliferation and transformation.

poly-A tail Polyadenylic acid sequence of varying length found at the 3' end of most eukaryotic mRNAs. Histone mRNAs do not have poly-A tail. The poly-A tail is added post-transcriptionally to the primary transcript as part of the nuclear processing of RNA yielding ***hnRNAs*** with 60–200 adenylate residues in the tail. In the cytoplasm the poly-A tail on mRNAs is gradually reduced in length. The function of the poly-A tail is not clear but it is the basis of a useful technique for the isolation of eukaryotic mRNAs. The technique uses an ***affinity column*** with oligo(U) or oligo(dT) immobilised on a solid support. If cytoplasmic RNA is applied to such a column, poly-A-rich RNA (mRNA) will be retained.

poly-A See ***polyadenylic acid***.

polyacrylamide gel electrophoresis (PAGE) Analytical and separative technique in which molecules, particularly proteins, are separated by their different ***electrophoretic mobilities*** in a hydrated gel. The gel suppresses convective mixing of the fluid phase through which the electrophoresis takes place, and contributes molecular sieving. Commonly carried out in the presence of the anionic detergent sodium dodecylsulphate (SDS). SDS denatures proteins so that non-covalently associating subunit polypeptides migrate independently, and by binding to the proteins confers a net negative charge roughly proportional to the chain weight. See also ***SDS-PAGE***.

polyadenylic acid ***Polynucleotide*** chain consisting entirely of residues of adenylic acid (i.e. the base sequence is AAAA....AAAA). Polyadenylic chains of various lengths are found at the 3' end of most eukaryotic mRNAs, the ***poly-A tail***.

polyanion Macromolecule carrying many negative charges. The commonest in cell biological systems is nucleic acid.

polycation Macromolecule with many positively charged groups. At physiological pH the most commonly used in cell biology is poly-L-lysine; this is often used to coat surfaces, thereby increasing the adhesion of cells (which have net negative surface charge). See also ***cationised ferritin***.

polycistronic mRNA A single ***mRNA*** molecule that is the product of the ***transcription*** of several tandemly arranged genes; typically the mRNA transcribed from an ***operon***.

polyclonal antibody An antibody produced by several clones of B-lymphocytes as would be the case in a whole

animal. Usually refers to antibodies raised in immunised animals, whereas a ***monoclonal antibody*** is the product of a single clone of B-lymphocytes, usually maintained *in vitro*.

polyclonal compartment When the progeny of several cells occupy an area or volume with a defined boundary, it is referred to as a polyclonal compartment, e.g. clones lying close to the mid-line of the wing of *Drosophila*.

polycloning site (multiple cloning site, MCS) Region of a phage or plasmid vector that has been engineered to contain a series of restriction sites that are usually unique within the entire vector. This makes it particularly easy to insert or excise (subclone) DNA fragments.

polycythaemia Increase in the haemoglobin content of the blood, either because of a reduction in plasma volume or an increase in red cell numbers. The latter may be a result of abnormal proliferation of red cell precursors (polycythaemia vera, Vaquez–Osler disease).

polyelectrolyte An ion with multiple charged groups.

polyendocrine syndrome Autoimmune disorder (the antigen to which the response is mounted is in the B-cells of the pancreas) in which there is involvement of several organ systems.

polyethylene glycol (PEG) A hydrophilic polymer that interacts with cell membranes and promotes fusion of cells to produce viable hybrids. Often used in producing ***hybridomas***.

polygalacturonan Plant cell wall polysaccharide consisting predominantly of galacturonic acid. May also contain some rhamnose, arabinose and galactose. Those with significant amounts of rhamnose are termed ***rhamnogalacturonans***. Found in the ***pectin*** fraction of the wall.

polygalacturonase Enzyme that degrades ***polygalacturonan*** by hydrolysis of the glycosidic bonds that link galacturonic acid residues. Important in fruit ripening and in fungal and bacterial attack on plants.

polyisoprenylation See ***geranoylation***.

polylysine A polymer of ***lysine***, it carries multiple positive charges and is used to mediate adhesion of living cells to synthetic culture substrates, or of fixed cells to glass slides (for observation by fluorescence microscopy, for example).

polymer A macromolecule made of repeating (monomer) units or ***protomers***.

polymerase chain reaction (PCR) The first practical system for *in vitro* amplification of DNA, and as such one of the most important recent developments in molecular biology. Two synthetic oligonucleotide primers, which are complementary to two regions of the target DNA (one for each strand) to be amplified, are added to the target DNA (which need not be pure) in the presence of excess deoxynucleotides and *Taq* polymerase, a heat-stable DNA polymerase. In a series (typically 30) of temperature cycles, the target DNA is repeatedly denatured (around 90°C), annealed to the primers (typically at 50–60°C) and a daughter strand extended from the primers (72°C). As the daughter strands themselves act as templates for subsequent cycles, DNA fragments matching both primers are amplified exponentially, rather than linearly. The original DNA need thus be neither pure nor abundant, and the PCR reaction has accordingly become widely used not only in research, but in clinical diagnostics and forensic science.

polymerisation The process of polymer formation. In many cases this requires ***nucleation*** and will occur only above a certain critical concentration.

polymorphic epithelial mucin See ***episialin***.

polymorphism 1. The existence, in a population, of two or more alleles of a gene, where the frequency of the rarer alleles is greater than can be explained by recurrent mutation alone (typically greater than 1%). HLA alleles of the ***major histocompatibility complex*** are very polymorphic. 2. The differentiation